Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China

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Abstract

Due to the well-known endocrine disrupting ability of bisphenol A (BPA), its production and usage have been regulated. Consequently, other bisphenol analogues (BPs) have been used as the replacement of BPA. Despite their widespread use, few studies have investigated the occurrence and fate of BPs in wastewater treatment plants (WWTPs). In this study, we conducted a city-wide monitoring survey and collected the wastewater and sludge samples from seven WWTPs in Xiamen, China over seven days. The concentrations of dissolved and adsorbed BPs in the influent and effluent, together with the BP concentrations in the sludge were determined. Five BPs, including BPA, BPAF, BPE, BPF, and BPS, were widely detected. The medium concentrations of BPA, BPAF, BPE, BPF, and BPS were 1318, 0.282, 3.70, 50.0, and 48.0 ng/L in the influent, 177, 0.714, 3.64, BLD, and BLD in the effluent, and 343, 3.09, BLD, 56.5, 1.01 $\mu\text{g/kg}$ in the sludge. Spatial variations were observed, which implied the industrial origin of BPA. The efficiencies of BP removal from aqueous phase were evaluated, and results showed that BPA, BPF, and BPS were highly removed with removal efficiencies higher than 78%, while BPAF and BPE were resistant in WWTPs. Mass load calculations showed that 2075 g BPs entered into the WWTPs in Xiamen City each day, while 246 g and 63 g were discharged via effluent and excess sludge, respectively. Mass balance analysis showed that BPS and BPA were mainly biodegraded, BPF were mainly adsorbed and biodegraded, BPAF was resistant to biodegradation, while BPE was resistant to both biodegradation and adsorption.

Keywords

Bisphenol analogues; Wastewater; Occurrence; Fate; Mass balance; Mass loads

Capsule Abstract

During wastewater treatment processes, BPA, BPF, and BPS were removed mainly via biodegradation, while BPE and BPAF were resistant to biodegradation.

1 Introduction

Bisphenol analogues (BPs) are a class of organic chemicals with two hydroxyphenyl functional group, including bisphenol A (BPA), bisphenol AF (BPAF), bisphenol B (BPB), bisphenol E (BPE), bisphenol F (BPF), bisphenol S (BPS), bisphenol Z (BPZ), and so on. BPs have a variety of industrial usage; for example, BPA is widely used to manufacture polycarbonate plastics and epoxy resins, BPAF is used to produce membranes and optical fibers, BPS is used in the thermal paper, and BPF, BPS, and BPAF are the main substitutes of BPA in the manufacturing of polycarbonate plastics and epoxy resins (Delfosse et al. 2012, Chen et al. 2016). Due to the well-known endocrine disrupting activity of BPA and other BPs (Delfosse et al. 2012, Rochester and Bolden 2015), great concerns have been raised on adverse effects of BPs on the human health and ecological safety.

Quite a few studies have investigated the occurrence and fate of BPA in the wastewater treatment plants (WWTPs) (Huang et al. 2012, Jiang et al. 2013, Mohapatra et al. 2010, Luo et al. 2014, Qiang et al. 2013, Carvalho et al. 2016, Belhaj et al. 2015). Results showed that BPA have been widely detected in the wastewater

and sludge, with the concentrations of ng/L-mg/L and $\mu\text{g/kg}$ -mg/kg, respectively (Huang et al. 2012, Jiang et al. 2013, Luo et al. 2014, Sun et al. 2014, Sun et al. 2016a). However, the knowledge on the occurrence of BPs other than BPA in the wastewater and sludge is limited. Researchers investigated sludge samples in Korea (Lee et al. 2015), US (Yu et al. 2015), and China (Song et al. 2014), respectively, which have provided fundamental data for the understanding of BP occurrence in the sludge (Chen et al. 2016). However, the information on the occurrence of BPs (other than BPA) in the wastewater either in the dissolved form or adsorbed onto the suspended solids (SS), and the integrated information of BPs in both wastewater and sludge, is limited.

WWTPs could partially remove BPA with the removal efficiencies of 62.5-99.6% (Luo et al. 2014). The aqueous phase removal percentage is often used to evaluate the removal efficiencies of BPA during the treatment process (Luo et al. 2014). However, the removal percentage based on the dissolved phase cannot comprehensively show the fate of organic pollutants in the WWTPs (Zhou et al. 2013), because BPs might adsorb onto the sludge/suspended particles due to their high values of $\log K_{ow}$ (Table S1, in the Supplemental Information, SI). As an alternative, mass balance assessment is a valuable way for understanding the fate of organic micropollutants and their mass loads to the receiving environments (Zhou et al. 2013, Supowit et al. 2016). So far, limited studies, including Bertanza et al (Bertanza et al. 2011), Guerra et al (Guerra et al. 2015), and Boni et al (Boni et al. 2012) have investigated the mass balance of BPA and provided useful information. However, the

studies of the fate of BPs other than BPA, including their removal efficiencies during wastewater treatment processes and their mass loads via effluent and sludge, was not yet available.

In this study, we collected the influent, effluent, and sludge samples from seven WWTPs in Xiamen, which is the major city in Southeast China. Seven BP concentrations in the wastewater, suspended solids (SS), and sludge were determined. The spatial and temporal variations of BP occurrence and removal were investigated. Special emphasis was placed on the mass balance assessment and mass load analysis of BPs during the wastewater treatment processes. The focus of this paper is to fill the knowledge gap of the fates of BPs in the WWTPs.

2 Materials and methods

2.1. Chemicals

BP compounds and bisphenol A isotope standards were purchased from Dr. Ehrenstorfer GmbH (Germany) and CNW (China). Physicochemical properties, including the molecular structure and log K_{ow} are given in Table S1 of SI. Methanol, acetonitrile and acetone (HPLC grade) were provided by Tedia (USA), $Na_4EDTA \cdot 2H_2O$ (analytical grade) was provided by Sinopharm (China). The reagent water was prepared with a Milli-Q water purification system (Millipore, USA). Stock solutions of individual BPs were prepared in methanol and stored at $-20\text{ }^{\circ}\text{C}$ in the dark.

2.2. Sampling campaign

Xiamen is a major city in the southeast of China (117°53'-118°25' E and 24°25'-24°54' N). The land area is 1573 km², and the population is 3.86 million in 2015, which showed a very high population density. There are seven steadily running WWTPs which receive both domestic wastewater and industrial wastewater, and another two small scale WWTPs which are under test run. Samples were collected from all the seven steadily running WWTPs (W1-W7) on February 28th to March 4th and March 6th, 2016. Automatic samplers were deployed at the influent, effluent, and return sludge (activated sludge in W1) to collect 24 h composite samples (0:00-24:00). The treatment processes, average daily processing capacity, average dry sludge production, and the ratio of domestic wastewater to industrial wastewater over the 7 sampling days are shown in Table 1. Detail performance of WWTPs on each sampling day is shown in Table S2 in SI. To further understand the contribution of individual treatment process on the BP removals, composite samples across the treatment processes in W3 were collected manually by taking 200 mL sample after every 2 hours from 10:00 a.m. to 10:00 a.m. the next day. Samples included the initial influent, the influent of anaerobic-anoxic-oxic (A2O) process, wastewater between the anaerobic and anoxic processes, wastewater between anoxic and oxic processes, wastewater after oxic process, final effluent, and return sludge on February 29th to March 6th 2016. After everyday sample collection, all the samples were immediately transferred to lab in the pre-cleaned glass bottles in the dark ice cooler, and the aqueous sample preparations were performed within 48 h.

2.3. Sample analysis

Wastewater samples were prepared using solid phase extraction (SPE) (Sun et al. 2014). Briefly, samples were filtered (GF/F glass fiber filters, 0.7 μm , Whatman), acidified to pH 2 using hydrochloric acid, added with 125 mg $\text{Na}_4\text{EDTA}\cdot 2\text{H}_2\text{O}$, and spiked with 50 μL isotope labelled standards as surrogate (500 $\mu\text{g/L}$). Oasis HLB cartridge (500 mg 6 mL, Waters) were preconditioned sequentially with 20 mL methanol, 6 mL water, and 6 mL water with pH 2.0, and then loaded by 500 mL samples at 5 mL/min. After the cartridges drying, analytes were eluted with 12 mL of methanol and 6 mL of mixture (acetone: methanol = 1:1). The eluates were evaporated to dryness with nitrogen stream and reconstituted to 1.00 mL with methanol. The samples were stored at 4 $^{\circ}\text{C}$ in the dark until the sample preparation was performed, which was within 48 h after sample collection.

Sludge and SS samples were prepared following the US EPA method 1694 (USEPA). Briefly, lyophilized samples (up to 0.100 g) were first extracted twice using mixture of 15.0 mL phosphate buffer (pH = 2.0) and 20 mL acetonitrile followed by extraction with 15.0 mL acetonitrile. The combined extract was then concentrated to 20-30 mL by rotary evaporation, and diluted with 200 mL of reagent water. Samples were then cleaned up via the SPE method used for liquid samples.

Instrumental analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Chromatographic separation of analytes was performed using a Kinetex C18 column (100 mm x 4.6 mm, 2.6 μm , Phenomenex, USA) by a

Shimadzu LC system (Shimadzu, Japan). A binary gradient (Table S3) with a flow rate of 0.80 mL/min was used. Mobile phase A contained 5 mmol/L ammonium acetate in water, while mobile phase B was methanol. The mass spectrometric measurements were carried out on an ABI triple quadrupole (QqQ) MS in the negative mode. The multiple reaction monitoring (MRM) mode was applied with the monitored target, reference ions, and other detail MS parameters shown in SI Table S1. The quantification was achieved via standard calibrations as shown in Table S4.

2.4. Quality assurance and quality control (QA/QC)

QA/QC was conducted to ensure the identification and quantification of the BPs. Identification of each BP was performed by LC-MS/MS with MRM, using the 2 highest characteristic precursor ion/product ion transition pairs (SI Table S1). The ratios of product transitions were calculated to ensure correct identification. Method quantification limits (MQLs), which were evaluated based on instrument quantification limits and analyte recovery (Li et al. 2016), were in the range of 0.043-2.43 ng/L for wastewater and 0.188-7.28 µg/kg for sludge (SI Table S1). An instrumental blank, procedural blank, sample duplicate, blank spike, and matrix spike, was applied for each batch. None of the target has been detected in the instrument blank and procedural blank, indicating the absence of the contamination. The average recovery of the isotopic standard was 85.3% and 120% for wastewater and sludge, respectively. The recoveries of the BPs were in the range of 43.1-89.8% for the wastewater, and 40.1-128% for the sludge, which fulfill the requirement for

simultaneous analysis of diverse targets in these challenging matrices (Guerra et al. 2014). Details are provided in Table S1.

2.5 Data analysis

Mass load of each BP in sludge, influent, and effluent was calculated according to Eq. (1-3). We applied a steady-state assumption (Supowit et al. 2016), which assumed the influent was equal to the load from discharge sources (effluent and sludge), and the losses via transit or volatility was negligible. Thus, the mass balance was performed via Eq. (4) and mass loss portion was calculated via Eq. (5). The removal efficiency of each BP from the aqueous phase during the treatment process was calculated using Eq. (6).

$$M_{\text{sludge}} = C_{\text{sludge}} \times Q_{\text{sludge}} \quad (1)$$

$$M_{\text{influent}} = C_{\text{influent(aqueous)}} \times Q_{\text{wastewater}} + C_{\text{influent(ss)}} \times Q_{\text{wastewater}} \times C_{\text{SS}} \quad (2)$$

$$M_{\text{effluent}} = C_{\text{effluent(aqueous)}} \times Q_{\text{wastewater}} + C_{\text{effluent(ss)}} \times Q_{\text{wastewater}} \times C_{\text{SS}} \quad (3)$$

$$M_{\text{loss}} = \sum M_{\text{influent}} - \sum M_{\text{effluent}} - \sum M_{\text{sludge}} \quad (4)$$

$$M_{\text{loss}}\% = (M_{\text{loss}}/M_{\text{influent}}) \times 100 \quad (5)$$

$$R(\%) = \frac{C_{\text{influent}} - C_{\text{effluent}}}{C_{\text{influent}}} \times 100 \quad (6)$$

Where C_{sludge} , $C_{\text{influent(aqueous)}}$, and $C_{\text{effluent(aqueous)}}$ are the BP concentrations in the sludge (ng/g, dw), influent (dissolved phase, ng/L), and effluent (dissolved phase, ng/L), respectively. $C_{\text{influent(ss)}}$ and $C_{\text{effluent(ss)}}$ are the adsorbed BP concentrations on

the SS from influent (ng/g) and effluent (ng/g). Q_{sludge} and $Q_{\text{wastewater}}$ are the processing capacity of sludge and wastewater in each WWTP (m^3/d). M_{sludge} , M_{influent} , and M_{effluent} are the mass load (g/d) of individual BP in the sludge, influent, and effluent. C_{ss} is the suspended solid content in the influent or effluent. $\sum M$ is the summation of mass loads in seven WWTPs.

Spearman's rank correlation analysis was conducted by PAST v 3.11 to evaluate the correlation among BPs. Principal component analysis (PCA) was conducted to analyze the spatial distribution behavior of BPs in influent, effluent, and sludge. In addition, Mann-Whitney pairwise comparison was applied to compare the BP concentrations and BP removal efficiencies among different WWTPs.

3 Results and discussions

3.1 Occurrence of BPs in WWTPs

Among seven targeted BPs, BPA, BPAF, BPE, BPF, and BPS were detected in wastewater, SS, and sludge. However, BPB and BPZ were below the method detection limits (BDLs) in all the samples. The concentration ranges, median, and geometric mean of the detected BPs in the influent, effluent, and sludge based on the each day sampling are shown in Table 2 and Figure 1. The average concentrations of dissolved BPA, BPAF, BPE, BPF, and BPS were 1960, 0.901, 4.51, 35.8, and 55.7 ng/L in the influent, 231, 1.16, 4.07, 1.67 and 0.596 ng/L in the effluent, while 477, 7.45, BDL, 70.9, and 2.25 ng/g in the sludge, respectively. The BP levels in the sludge were within the similar ranges as those in Korea (Lee et al. 2015), USA (Yu et

al. 2015), and China (Song et al. 2014). The significantly high concentration of BPA indicated that BPA was the most widely used BPs in Xiamen, China. In addition, Spearman's test (r_s) showed that there was significant positive correlation among BPA, BPF, and BPS ($p < 0.01$, $0.40 < r < 0.67$), and between BPE and BPAF ($p = 0.0088$, $r = 0.37$) in the influent, indicating similar discharge pattern.

3.2 Temporal and spatial variations of BPs in WWTPs

Temporal variations were observed in influent, effluent and sludge (Fig. 1) among seven sampling days, although the 24 h composite sampling strategy applied. The variations in the influent were probably because of the fluctuations of BP discharge from the industrial and domestic wastewater, while the variations in the effluent might be due to the combination effect of the influent and the removal efficiencies. The temporal variations indicated the sampling over an extended period of time is necessary for the BP investigations.

BP concentrations showed spatial variations among WWTPs. PCA result showed the dissolved BP concentrations in the influent could be clustered into separate groups among different WWTPs (Fig. 2). Samples from W1 were separated due to the lower concentrations of BPA, BPF, and BPS (Mann-Whitney pairwise comparison, p values are shown in SI Table S5). Samples from W2 were with significantly high concentrations of BPA and BPS (SI Table S5). Samples from W3 were with low concentrations of BPA and BPS, and high concentrations of BPAF (SI Table S5). Samples from W4 were with significantly high concentrations of BPS and high

concentrations of BPA (SI Table S5). Samples from W7 were with significantly high concentrations of BPE ($p<0.01$).

The spatial variations in the influent were probably related to the wastewater source. Previous studies showed that BPA concentrations were higher in the industrial wastewater (Huang et al. 2012, Sun et al. 2016a, Lee et al. 2015) because it is an industrially important chemical and mainly used in the manufacture of plastics related products (Huang et al. 2012). In this study, W1 and W3 mainly receive the domestic wastewater, while W4-7 receive at least 50% of the industrial wastewater (Table 1), which might contain higher BPA. An exception was observed in W2, where higher BPA was detected in spite of only 10% industrial wastewater contribution. According to Yearbook of Xiamen Special Economic Zone (2015), the annual industrial output value from enterprise above designated size was high in W2 served area. Spearman's test (r_s) showed the positive correlation ($p=0.023$, $r=0.85$) between BPA concentrations and the annual industrial output value above designated size, which confirmed BPA's industrial origin.

In addition, the spatial variations of BPs were also observed in the effluent and sludge (Figure S1 in SI). For example, the sludge samples collected from W2, W4 and W6 showed significantly high levels of BPAF, BPA and BPF ($p<0.01$, Mann-Whitney test), respectively, and the effluent collected from W2, W3 and W7 showed significantly high levels of BPA, BPAF, and BPE ($p<0.01$, Mann-Whitney test).

3.3 Removal efficiencies of dissolved BPs in the WWTPs

Fig. 3 shows the removal efficiencies of dissolved BPs in W1-W7. The wastewater treatment processes showed the highest removal of BPS with the mean removal efficiencies of 98.9% in the 7 WWTPs during the 7-day sampling, followed by BPF and BPA, with the values of 93.8% and 78.3%, respectively. Previous studies showed that biological and chemical degradation, as well as adsorption onto the sludge, were the major contribution to dissolved organic micropollutant removal during the wastewater treatment processes (Qiang et al. 2013, Sun et al. 2016a). To further understand the contribution of BP removals, composite samples of initial influent, influent of A2O process, wastewater between the anaerobic and anoxic processes, wastewater between anoxic and oxic processes, wastewater after oxic process, and final effluent were collected in W3 from February 29th to March 6th 2016. The removal contributions of BPA, BPAF, BPF, and BPS are shown in Fig. 4, while BPE was not included due to the low detection frequencies to run data analysis. The anaerobic process contributed most for BPA, BPF, and BPS removal, with the average efficiencies of 56.0%, 62.1%, and 33.0%, followed by the treatment in the screen and grit chamber, with the average efficiencies of 12.2%, 17.6%, and 23.9%, respectively. The oxic process also removed 10.0% of BPA, 5.3 % of BPF, and 23.2% of BPS, while the anoxic process contribute minor (<10%) on these three BPs removal. The rapid decrease of BPA, BPF, and BPS in the anaerobic processes was probably due to the dilution via the return sludge flow, adsorption via the activated sludge, and anaerobic biodegradation (Xue et al. 2010, Avila et al. 2013). In addition, Avila et al showed that compared to the anaerobic treatment, the aerobic

biodegradation was the major removal pathway for BPA (Avila et al. 2013). Chang et al showed the aerobic biodegradation rate of BPF higher than BPA (Chang et al. 2014). Therefore, the removal of BPA and BPF in the oxic tank seemed to be due to the aerobic biodegradation.

Negative removal efficiencies were observed for BPAF (-153%) and BPE (-82.5%), indicating that their concentrations in the effluent was higher than influent. Previous studies of wastewater monitoring also showed the negative removal efficiencies of some emerging contaminants during wastewater treatment processes, including but not limited to triclosan (Sun et al. 2014, Sun et al. 2016a), triclocarban (Sun et al. 2014, Sun et al. 2016a), propranolol (Sun et al. 2014, Maurer et al. 2007), carbamazepine (Sun et al. 2014, Clara et al. 2005, Clara et al. 2004). The proposed reasons for the negative values were: (1) concentration fluctuations caused by the sampling strategy (Madrid and Zayas 2007, Ort et al. 2010), (2) concentration shift in the influent and effluent due to the hydraulic retention time (HRT) (Rodayan et al. 2014), (3) analyte released from the adsorbed phase (Lindberg et al. 2005), (4) lack of the degrading bacteria in the microbial community of the activated sludge, and (5) analyte transformed from the conjugated forms. The grab sampling strategy, which only reflected the concentrations at the moment of sampling (Madrid and Zayas 2007), might lead to the uncertainty of analyte concentrations (Ort et al. 2010). In addition, the HRT might cause the concentration shift in the influent and effluent (Rodayan et al. 2014). However, in this study, 24 h composite samples minimize the fluctuations of BP concentrations, and the average concentrations obtained from the consistent

sampling over seven days reduce the concentrations shift due to HRT. Thus, the fluctuation due to the sampling strategy was excluded. Furthermore, analyte might release from the adsorbed phase via return sludge due to the changes in physicochemical parameters during the treatment processes (Lindberg et al. 2005). As shown in Fig. 5, the mass portions of BPAF and BPE in the sludge were 33.7% and 1.1% respectively, which indicate the minor contribution of released BPs via the return sludge with the returned sludge ratio in the range of 50-200% in the seven WWTPs. Previous studies ranked the biodegradability of BPs as BPF> BPA> BPE (Ike et al. 2006, Sakai et al. 2007), indicating low biodegradability of BPE. Although the information on the BPAF biodegradation is limited, there is evidence that BPAF has higher environmental persistence than the other BP analogues (Chen et al. 2016). Thus, the lack of BPAF and BPE degrading bacteria in the activated sludge and their robust structure might cause the low removal efficiencies. In addition, the human excrete BPA as BPA conjugates, mainly in glucuronide and sulfate form (Chen et al. 2016). Although the information on conjugates of other BP analogues and their fate during the wastewater treatment processes is limited, the study on the estrogen conjugates showed their short half-life in the sewage and the conjugates were mainly transformed to the free estrogens (Kumar et al. 2012), indicating the possibility of transformation from BP conjugates to the free BPs. Thus, the conjugated BPs, which were not detected in the influent, might be transformed to the free BPs via the biological processes (Blair et al. 2015, Liao and Kannan 2012, Liu et al. 2015), and accordingly increased BP concentrations in the effluent. Therefore, the negative

removal values of BPAF and BPE were probably due to their robust structure, lack of degrading bacteria, and the release from the conjugate forms.

To be noticed, although the treatment processes are different among the seven WWTPs, the consistent high removal efficiencies of BPA, BPF, and BPS, together with the consistent low removal efficiencies of BPAF and BPE over 7 days indicate that BPs can be applied as sewage markers to designate the direct discharge of untreated wastewater into the surface water (Lv et al. 2014, Nakada et al. 2008, Sun et al. 2016b). As discussed in Section 3.2, due to the strong evidence on BPA's industrial origin, BPA can be used as the industrial sewage marker.

3.4 Mass balance

Mass loads of each BP in the seven WWTPs based on each day sampling are shown in Table 3. BPA was the predominant BP. There was 1969 g BPA entered the WWTPs via influent each day in Xiamen, equaled to 0.510 mg/capita, which was mainly dissolved in the aqueous phase (1950 g). The effluent and excess sludge from the 7 WWTPs discharged 232 g and 55.1 g BPA to the receiving environment. The effluent was the major pathway of BPA to the environment.

The total mass loads of BPAF, BPE, BPF, and BPS in influent corresponded to 2.35, 6.44, 41.2, and 56.2 g, with the dissolved mass values of 1.29, 1.94, 5.32, and 1.19 g, respectively. After the treatment processes, the total mass loads of BPAF, BPE, BPF, and BPS were 1.97, 4.12, 7.21, and 0.703 g in the effluent, and were 0.813, 0.0228, 7.21, and 0.259 g in the sludge, respectively.

Mass balance was performed to investigate the fate of BPs during the wastewater treatment processes. As shown in Fig. 5, 98.3% of BPS, 80.7% of BPA, and 61.7% of BPF were removed. In the case of BPE, 26.6% was removed and 73.1% was discharged via the effluent. The mass loads of BPAF in the sludge and effluent were even higher than those in the influent, indicating that BPAF was recalcitrant during the wastewater treatment processes. Since the adsorbed BPs onto the sludge or SS were evaluated in the mass balance analysis, the mass loss was mainly attributed to the biodegradation (Xue et al. 2010). Therefore, BPS and BPA were mainly removed through biodegradation, BPF was mainly removed through combined sorption-biodegradation, BPE was resistant to both biodegradation and adsorption, while BPAF was resistant to biodegradation.

4 Conclusions

This study investigated the occurrence and fate of BPs in WWTPs, and closed the balance by determining the BP concentrations in the influent, effluent, and sludge. BPs were widely detected in the influent, effluent, and sludge. The concentrations ranked as $BPA \gg BPS \approx BPF > BPE > BPAF$ in the influent, $BPA \gg BPE > BPF \approx BPAF > BPS$ in the effluent, and $BPA \gg BPF > BPAF > BPS > BPE$ in the sludge. Spatial and temporal variations were observed. Based on the wastewater source and the annual industrial output value in each WWTP served area, BPA showed strong industrial origin.

Wastewater treatment processes could partially remove BPs. BPA, BPF, and BPS

was highly removed from the aqueous phase. However, BPAF and BPE were recalcitrant in WWTPs . In addition, the mass balance analysis was performed and results indicated that BPS and BPA were mainly biodegraded during the wastewater treatment processes, BPF was mainly biodegraded and adsorbed on the sludge, BPAF was resistant to biodegradation, while BPE was resistant to both biodegradation and adsorption. The mass load calculations showed that 2075 g BPs enter into the WWTPs via influent in Xiamen each day, while 246 g and 63 g were discharged via effluent and excess sludge, respectively. Due to the endocrine disrupting activity of BPs, the potential risk of discharged BPs via both effluent and sludge should be assessed.

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Figure captions:

Fig. 1 BP concentrations in the influent, effluent, and sludge, where error bars presents the minimum to maximum (n=7)

Fig. 2 Spatial variations of BP concentrations in influent

Fig.3 BP removal efficiencies from the aqueous phase among W1-W7

395 Fig. 4 BPs removal contributions of each wastewater treatment process in W3, where
396 error bars presents the minimum to maximum (n=7)

397 Fig. 5 Mass portion of the detected BPs in the effluent, sludge, and the total loss
398 during the treatment processes

399 Fig. 6 Average daily mass loads over 7 days, where error bars presents the standard
400 deviation (n=7)

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Reference

- Delfosse, V., Grimaldi, M., Pons, J.L., Boulahtouf, A., le Maire, A., Cavailles, V., Labesse, G., Bourguet, W. and Balaguer, P. (2012) Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes. *Proc Natl Acad Sci U S A* 109(37), 14930-14935.
- Chen, D., Kannan, K., Tan, H.L., Zheng, Z.G., Feng, Y.L., Wu, Y. and Widelka, M. (2016) Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity-A Review. *Environmental science & technology* 50(11), 5438-5453.
- Rochester, J.R. and Bolden, A.L. (2015) Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environmental Health Perspectives* 123(7), 643-650.
- Huang, Y.Q., Wong, C.K.C., Zheng, J.S., Bouwman, H., Barra, R., Wahlstrom, B., Neretin, L. and Wong, M.H. (2012) Bisphenol A (BPA) in China: A review of sources, environmental levels, and potential human health impacts. *Environment International* 42, 91-99.
- Jiang, J.Q., Zhou, Z. and Sharma, V.K. (2013) Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water - A review from global views. *Microchemical Journal* 110, 292-300.
- Mohapatra, D.P., Brar, S.K., Tyagi, R.D. and Surampalli, R.Y. (2010) Physico-chemical pre-treatment and biotransformation of wastewater and wastewater Sludge - Fate of bisphenol A. *Chemosphere* 78(8), 923-941.
- Luo, Y.L., Guo, W.S., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S. and Wang, X.C.C. (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment* 473, 619-641.
- Qiang, Z.M., Dong, H.Y., Zhu, B., Qu, J.H. and Nie, Y.F. (2013) A comparison of various rural wastewater treatment processes for the removal of endocrine-disrupting chemicals (EDCs). *Chemosphere* 92(8), 986-992.
- Carvalho, A.R., Cardoso, V., Rodrigues, A., Benoliel, M.J. and Duarte, E. (2016) Fate and Analysis of Endocrine-Disrupting Compounds in a Wastewater Treatment Plant in Portugal. *Water Air and Soil Pollution* 227(6).
- Belhaj, D., Baccar, R., Jaabiri, I., Bouzid, J., Kallel, M., Ayadi, H. and Zhou, J.L. (2015) Fate of selected estrogenic hormones in an urban sewage treatment plant in Tunisia (North Africa). *Science of the Total Environment* 505, 154-160.
- Sun, Q., Lv, M., Hu, A.Y., Yang, X.Y. and Yu, C.P. (2014) Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China. *Journal of Hazardous Materials* 277, 69-75.
- Sun, Q., Li, M.Y., Ma, C., Chen, X.Q., Xie, X.Q. and Yu, C.P. (2016a) Seasonal and spatial variations of PPCP occurrence, removal and mass loading in three wastewater treatment plants located in different urbanization areas in Xiamen, China. *Environmental Pollution* 208, 371-381.
- Lee, S., Liao, C., Song, G.J., Ra, K., Kannan, K. and Moon, H.B. (2015) Emission of bisphenol analogues including bisphenol A and bisphenol F from wastewater treatment plants in Korea. *Chemosphere* 119, 1000-1006.
- Yu, X.H., Xue, J.C., Yao, H., Wu, Q., Venkatesan, A.K., Halden, R.U. and Kannan, K. (2015) Occurrence and estrogenic potency of eight bisphenol analogs in sewage sludge from the US EPA targeted national sewage sludge survey. *Journal of Hazardous Materials* 299, 733-739.
- Song, S.J., Song, M.Y., Zeng, L.Z., Wang, T., Liu, R.Z., Ruan, T. and Jiang, G.B. (2014) Occurrence and

447 profiles of bisphenol analogues in municipal sewage sludge in China. *Environmental Pollution* 186,
448 14-19.

449 Zhou, L.J., Ying, G.G., Liu, S., Zhao, J.L., Yang, B., Chen, Z.F. and Lai, H.J. (2013) Occurrence and fate of
450 eleven classes of antibiotics in two typical wastewater treatment plants in South China. *Science of the*
451 *Total Environment* 452, 365-376.

452 Supowit, S.D., Sadaria, A.M., Reyes, E.J. and Halden, R.U. (2016) Mass Balance of Fipronil and Total
453 Toxicity of Fipronil-Related Compounds in Process Streams during Conventional Wastewater and
454 Wetland Treatment. *Environmental science & technology* 50(3), 1519-1526.

455 Bertanza, G., Pedrazzani, R., Dal Grande, M., Papa, M., Zambarda, V., Montani, C., Steimberg, N.,
456 Mazzoleni, G. and Di Lorenzo, D. (2011) Effect of biological and chemical oxidation on the removal of
457 estrogenic compounds (NP and BPA) from wastewater: An integrated assessment procedure. *Water*
458 *Research* 45(8), 2473-2484.

459 Guerra, P., Kim, M., Teslic, S., Alaei, M. and Smyth, S.A. (2015) Bisphenol-A removal in various
460 wastewater treatment processes: Operational conditions, mass balance, and optimization. *Journal of*
461 *Environmental Management* 152, 192-200.

462 Boni, M.R., Sbaffoni, S., Tedesco, P. and Vaccari, M. (2012) Mass balance of emerging organic
463 micropollutants in a small wastewater treatment plant. *Water Pollution Xi* 164, 345-356.

464 USEPA Method 1694: pharmaceuticals and personal care products in water, soil, sediment, and
465 biosolids by HPLC/MS/MS. Washington, DC: United States Environmental Protection Agency Office of
466 Water; 2007. EPA 821-R-08-002.

467 Li, M., Sun, Q., Li, Y., Lv, M., Lin, L., Wu, Y., Ashfaq, M. and Yu, C.-p. (2016) Simultaneous analysis of 45
468 pharmaceuticals and personal care products in sludge by matrix solid-phase dispersion and liquid
469 chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 1-12.

470 Guerra, P., Kim, M., Shah, A., Alaei, M. and Smyth, S.A. (2014) Occurrence and fate of antibiotic,
471 analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes.
472 *Science of the Total Environment* 473, 235-243.

473 (2015) Xiamen Statistical Bureau, Yearbook of Xiamen Special Economic Zone.

474 Xue, W.C., Wu, C.Y., Xiao, K., Huang, X., Zhou, H.D., Tsuno, H. and Tanaka, H. (2010) Elimination and
475 fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined
476 with membrane bioreactor for municipal wastewater reclamation. *Water Research* 44(20), 5999-6010.

477 Avila, C., Reyes, C., Bayona, J.M. and Garcia, J. (2013) Emerging organic contaminant removal
478 depending on primary treatment and operational strategy in horizontal subsurface flow constructed
479 wetlands: Influence of redox. *Water Research* 47(1), 315-325.

480 Chang, B.V., Liu, J.H. and Liao, C.S. (2014) Aerobic degradation of bisphenol-A and its derivatives in
481 river sediment. *Environmental Technology* 35(4), 416-424.

482 Maurer, M., Escher, B.I., Richle, P., Schaffner, C. and Alder, A.C. (2007) Elimination of beta-blockers in
483 sewage treatment plants. *Water Research* 41(7), 1614-1622.

484 Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N. and Kroiss, H. (2005) Removal of selected
485 pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and
486 conventional wastewater treatment plants. *Water Research* 39(19), 4797-4807.

487 Clara, M., Strenn, B. and Kreuzinger, N. (2004) Carbamazepine as a possible anthropogenic marker in
488 the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment
489 and during groundwater infiltration. *Water Research* 38(4), 947-954.

490 Madrid, Y. and Zayas, Z.P. (2007) Water sampling: Traditional methods and new approaches in water

sampling strategy. *Trac-Trends in Analytical Chemistry* 26(4), 293-299.

Ort, C., Lawrence, M.G., Reungoat, J. and Mueller, J.F. (2010) Sampling for PPCPs in Wastewater Systems: Comparison of Different Sampling Modes and Optimization Strategies. *Environmental science & technology* 44(16), 6289-6296.

Rodayan, A., Majewsky, M. and Yargeau, V. (2014) Impact of approach used to determine removal levels of drugs of abuse during wastewater treatment. *Science of the Total Environment* 487, 731-739.

Lindberg, R.H., Wennberg, P., Johansson, M.I., Tysklind, M. and Andersson, B.A.V. (2005) Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environmental science & technology* 39(10), 3421-3429.

Ike, M., Chen, M.Y., Danzl, E., Sei, K. and Fujita, M. (2006) Biodegradation of a variety of bisphenols under aerobic and anaerobic conditions. *Water Science and Technology* 53(6), 153-159.

Sakai, K., Yamanaka, H., Moriyoshi, K., Ohmoto, T. and Ohe, T. (2007) Biodegradation of bisphenol A and related compounds by *Sphingomonas* sp strain BP-7 isolated from seawater. *Bioscience Biotechnology and Biochemistry* 71(1), 51-57.

Kumar, V., Johnson, A.C., Nakada, N., Yamashita, N. and Tanaka, H. (2012) De-conjugation behavior of conjugated estrogens in the raw sewage, activated sludge and river water. *Journal of Hazardous Materials* 227, 49-54.

Blair, B., Nikolaus, A., Hedman, C., Klaper, R. and Grundl, T. (2015) Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. *Chemosphere* 134, 395-401.

Liao, C.Y. and Kannan, K. (2012) Determination of Free and Conjugated Forms of Bisphenol A in Human Urine and Serum by Liquid Chromatography-Tandem Mass Spectrometry. *Environmental science & technology* 46(9), 5003-5009.

Liu, Z.H., Lu, G.N., Yin, H., Dang, Z. and Rittmann, B. (2015) Removal of Natural Estrogens and Their Conjugates in Municipal Wastewater Treatment Plants: A Critical Review. *Environmental science & technology* 49(9), 5288-5300.

Lv, M., Sun, Q., Hu, A.Y., Hou, L.Y., Li, J.W., Cai, X. and Yu, C.P. (2014) Pharmaceuticals and personal care products in a mesoscale subtropical watershed and their application as sewage markers. *Journal of Hazardous Materials* 280, 696-705.

Nakada, N., Kiri, K., Shinohara, H., Harada, A., Kuroda, K., Takizawa, S. and Takada, H. (2008) Evaluation of pharmaceuticals and personal care products as water-soluble molecular markers of sewage. *Environmental science & technology* 42(17), 6347-6353.

Sun, Q., Li, Y., Li, M., Ashfaq, M., Lv, M., Wang, H., Hu, A. and Yu, C.P. (2016b) PPCPs in Jiulong River estuary (China): Spatiotemporal distributions, fate, and their use as chemical markers of wastewater. *Chemosphere* 150, 596-604.

Fig. 1 BP concentrations in the influent, effluent, and sludge, where error bars presents the minimum to maximum (n=7)

Fig. 2 Spatial variations of BP concentrations in influent

Fig.3 BP removal efficiencies from the aqueous phase among W1-W7

Fig. 4 BPs removal contributions of each wastewater treatment process in W3, where error bars presents the minimum to maximum (n=7)

Fig. 5 Mass portion of the detected BPs in the effluent, sludge, and the total loss during the treatment processes

Fig. 6 Average daily mass loads over 7 days, where error bars presents the standard deviation (n=7)

Table 1 The treatment processes, average daily processing capacity, and average dry sludge production

WWTPs	Treatment processes	Daily processing capacity ($\times 10^4$ t)	Daily dry sludge production (t)	Ratio of domestic to industrial wastewater
W1	primary sedimentation, biological aerated filters (BAF), UV disinfection	22.35	18.50	10:0
W2	primary sedimentation, oxidation ditches, secondary sedimentation, UV disinfection	18.07	35.22	9:1
W3	primary sedimentation, A2O, secondary sedimentation, UV disinfection	7.43	15.22	8:2
W4	primary sedimentation, A2/O, secondary sedimentation, chemical disinfection	5.65	19.82	5:5
W5	primary sedimentation, A2O, secondary sedimentation, UV disinfection	6.30	8.95	5:5
W6	primary sedimentation, oxidation ditches, secondary sedimentation, UV disinfection	8.24	13.85	5:5
W7	primary sedimentation, oxidation ditches, secondary sedimentation, UV disinfection	3.16	1.96	4:6

Table 2 The range and median values of bisphenol analogue concentrations in the wastewater (ng/L) and sludge (µg/kg, dw) in WWTPs (n=7 ^a)

WWT Ps	Type	BPA			BPAF			BPE			BPF			BPS		
		influent	effluent	sludge	influent	effluent	sludge	influent	effluent	sludge	influent	effluent	sludge	influent	effluent	sludge
W1	range	189-590	16.8-544	72.5-577	0.0562-0.576	0.119-0.852	1.92-3.09	BDL-10.4	BDL-2.22	BDL	BDL-16.3	BDL-4.90	BDL-47.1	BDL-24.6	BDL-0.600	BDL
	median	320	132	123	0.220	0.494	2.29	2.78	1.31	BDL	7.88	BDL	BDL	17.8	BDL	BDL
	GM	345	116	145	0.223	0.351	2.14	2.60	1.12	BDL	12.4	3.57	45.9	20.1	0.245	BDL
W2	range	1040-20400	460-1100	167-406	0.156-1.02	0.454-3.36	2.97-5.81	1.72-18.6	1.64-9.14	BDL	1.72-18.6	BDL-13.2	BDL-41.3	14.5-202	BDL-3.70	BDL-3.60
	median	7020	720	317	0.576	0.956	4.21	3.92	5.36	BDL	69.6	3.90	22.6	95.4	3.40	2.28
	GM	6030	696	311	0.475	1.03	4.17	3.97	4.34	BDL	78.7	7.13	33.3	81.9	3.08	2.18
W3	range	254-512	60.4-312	92.8-246	4.24-12.6	1.11-13.2	29.5-42.4	1.64-3.64	2.64-13.0	BDL	28.8-127	BDL-7.94	54.3-135	17.5-254	BDL-2.04	BDL-2.47
	median	462	172	180	10.8	10.14	30.7	2.88	3.28	BDL	62.4	BDL	85.0	26.0	BDL	BDL
	GM	400	147	169	8.91	6.88	32.3	2.33	3.90	BDL	62.1	7.94	86.2	23.5	0.390	1.47
W4	range	1160-6480	46-310	924-1980	BDL-0.488	BDL-16.6	0.804-2.54	BDL-5.48	BDL-15.7	BDL-7.99	31.8-93.6	BDL-4.66	62.2-106	183-746	BDL-0.902	BDL-3.77
	median	2080	172	1320	0.110	0.250	1.86	4.44	8.32	BDL	46.6	BDL	83.6	349	BDL	3.31
	GM	2210	147	1350	0.167	0.345	1.71	4.16	3.68	7.99	47.0	4.66	82.2	318	0.592	3.09
W5	range	494-1458	16.2-314	348-1630	0.110-1.02	BDL-0.878	2.17-5.13	BDL-5.42	1.32-3.26	BDL	9.06-79.6	BDL	44.2-75.1	26.2-115.4	BDL-1.32	BDL-2.29
	median	1090	88.4	531	0.194	0.466	3.09	1.62	1.63	BDL	13.7	BDL	52.6	43.2	0.0500	BDL
	GM	1050	158	478	0.281	0.419	3.13	1.60	3.97	BDL	17.8	0.972	52.3	41.8	2.42	1.68
W6	range	870-2420	62.6-566	292-531	BDL-0.370	0.248-0.756	2.91-4.82	1.32-8.12	1.83-8.54	BDL	25.2-166	BDL-1.972	53.5-336	51.6-111	BDL-2.42	BDL-12.2
	median	1360	218	384	0.144	0.398	3.68	4.70	4.28	BDL	64.8	BDL	127	96.8	BDL	6.77
	GM	1450	158	382	0.141	0.419	3.67	4.43	3.97	BDL	66.1	0.972	143	88.4	2.42	8.56
W7	range	590-5560	53.6-298	200-386	BDL-0.556	0.268-1.29	2.29-3.90	2.80-97.6	8.06-75.2	BDL	27.4-104	BDL-35.4	34.4-86.1	2.18-74.2	BDL-0.850	BDL
	median	2020	175	287	0.220	0.852	2.88	39.4	55.2	BDL	74.2	20.8	52.6	27.6	BDL	BDL
	GM	1960	144	281	0.284	0.730	2.89	30.8	39.9	BDL	69.9	21.6	49.9	25.8	0.265	BDL

“GM”, geometric mean, which was calculated based on the detected concentration; “BDL”, below detection limits; ^{“a”}, based on the samples collected from each day.

Table 3 Daily mass loads of BPs (mean± standard deviation, n=7^a, unit: g)

BPs	Sample type		W1	W2	W3	W4	W5	W6	W7	Sum
BPA	I	D	83.4±35.8	1420±1040	30.6±7.6	145±93	68.9±20.4	125±43	72.2±41.7	1950
		SS	4.47±7.88	5.87±4.38	1.21±1.22	4.20±1.15	1.14±0.63	1.90±1.78	0.460±0.409	19.3
	E	D	45.6±39.6	131±38	12.6±7.0	10.1±5.6	8.53±7.29	18.0±14.7	5.31±3.07	231
		SS	0.0609±0.1419	0.270±0.325	0.106±0.185	0.0689±0.0719	0.127±0.306	0.142±0.305	0.145±0.222	0.920
	S		3.00±1.09	11.0±3.18	2.69±0.92	27.9±8.6	4.39±1.06	5.43±1.56	0.701±0.233	55.1
BPAF	I	D	0.0639±0.0425	0.207±0.042	0.735±0.160	0.0136±0.0076	0.0227±0.0180	0.0138±0.0120	0.00836±0.00601	1.06
		SS	0.476±0.173	0.0127±0.0172	0.251±0.341	0.104±0.032	0.148±0.050	0.225±0.098	0.0763±0.0245	1.29
	E	D	0.0975±0.0640	0.225±0.175	0.624±0.290	0.127±0.310	0.0279±0.0217	0.0387±0.0187	0.0269±0.0136	1.17
		SS	0.212±0.193	0.187±0.128	0.151±0.054	0.0668±0.0641	0.0710±0.0464	0.0968±0.0654	0.0277±0.0143	0.812
	S		0.0438±0.0133	0.150±0.054	0.495±0.118	0.0364±0.0139	0.0289±0.0082	0.0515±0.0100	0.00847±0.00372	0.813
BPE	I	D	0.952±0.850	0.987±1.06	0.190±0.072	0.241±0.059	0.156±0.125	0.431±0.223	1.54±1.17	4.50
		SS	0.868±0.347	0.322±0.213	0.231±0.099	0.183±0.116	0.202±0.095	0.0806±0.1145	0.0550±0.0340	1.94
	E	D	0.336±0.246	0.946±0.557	0.347±0.273	0.395±0.339	0.128±0.059	0.368±0.190	1.55±0.85	4.07
		SS	0.00188±0.00498	0.0221±0.0481	0.0115±0.0180	0.00403±0.00798	0.00998±0.00264	0.00968±0.0256	0.00336±0.00548	0.0536
	S		NA	NA	NA	0.0228±0.0602	NA	NA	NA	0.0228
BPF	I	D	1.67±1.76	15.0±5.6	6.21±5.04	2.80±1.08	1.44±1.37	6.42±3.99	2.37±0.90	35.9
		SS	0.322±0.852	0.805±0.769	0.130±0.343	1.37±0.28	0.538±0.379	2.07±0.63	0.0778±0.2060	5.32
	E	D	0.239±0.426	0.817±0.925	0.0851±0.2251	0.0406±0.1074	NA	0.0115±0.0305	0.480±0.379	1.67
		SS	1.11±1.06	1.53±0.79	0.739±0.392	0.561±0.172	0.498±0.360	0.767±0.184	0.321±0.117	5.54
	S		0.465±0.646	0.722±0.81	1.35±0.46	1.67±0.44	0.410±0.203	2.43±1.67	0.161±0.083	7.21
BPS	I	D	3.94±1.88	18.1±10.0	1.79±0.39	19.7±10.0	2.92±1.66	7.48±1.73	0.959±0.588	55.0
		SS	0.162±0.084	0.259±0.215	0.0427±0.0458	0.388±0.506	0.0948±0.0287	0.214±0.062	0.0245±0.0233	1.19

	E	D	0.0241±0.0539	0.483±0.232	0.0280±0.0557	0.0102±0.0190	0.0138±0.0294	0.0297±0.0787	0.00741±0.01241	0.596
		SS	0.0243±0.0445	0.0329±0.0564	0.0130±0.0225	0.00950±0.0136	0.0124±0.0213	0.00955±0.02526	0.00517±0.01367	0.107
	S	NA		0.0755±0.0140	0.00328±0.00867	0.0617±0.0109	0.00422±0.00734	0.108±0.063	0.00624±0.01395	0.259

I, influent; E, effluent; S, sludge; D, dissolved BPs; SS, BPs adsorbed onto the suspended solids; “NA”, not available due to the target was not detected; “^{ca}”, based on the samples collected from each day.